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Article

Disposable Nafion-Coated Single-Walled Carbon Nanotube Test Strip for Electrochemical Quantitative Determination of Acetaminophen in a Finger-Prick Whole Blood Sample

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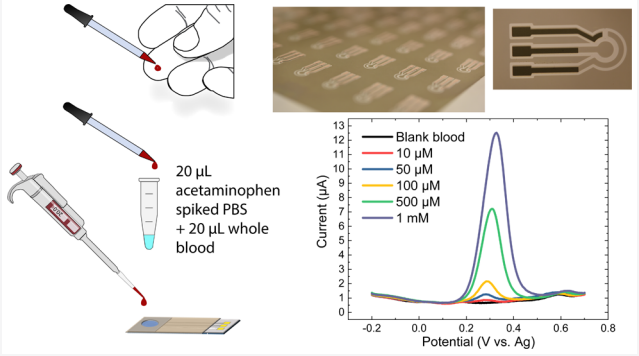
ABSTRACT: A disposable electrochemical test strip for the quantitative point-of-care (POC) determination of acetaminophen (paracetamol) in plasma and finger-prick whole blood was fabricated. The industrially scalable dry transfer process of single-walled carbon nanotubes (SWCNTs) and screen printing of silver were combined to produce integrated electrochemical test strips. Nafion coating stabilized the potential of the Ag reference electrode and enabled the selective detection in spiked plasma as well as in whole blood samples. The test strips were able to detect acetaminophen in small 40 μL samples with a detection limit of 0.8 μM and a wide linear range from 1 μM to 2 mM, well within the required clinical range. After a simple 1:1 dilution of plasma and whole blood, a quantitative detection with good recoveries of 79% in plasma and 74% in whole blood was achieved. These results strongly indicate that these electrodes can be used directly to determine the unbound acetaminophen fraction without the need for any additional steps. The developed test strip shows promise as a rapid and simple POC quantitative acetaminophen assay.



INTRODUCTION

Acetaminophen (paracetamol) is one of the most widely used analgesics with antipyretic properties.[1] It is readily available, inexpensive, and better-tolerated than nonsteroidal anti-inflammatory drugs (NSAIDs).[1] It is therefore widely recommended for the treatment of fever and pain.[2] Acetaminophen is also commonly prescribed in combination with opioids due to its opioid-sparing effect.[1] However, unlike NSAIDs, doses only slightly larger than recommended can cause hepatotoxicity. Acetaminophen is one of the most commonly overdosed drugs, and acetaminophen poisoning is currently the leading cause of acute liver failure in the United States and Europe.[3,4] In the United States alone, the poison control centers receive over 111 000 consultations related to acetaminophen and there are 40 000 associated emergency department cases annually.[5] Both intentional and unintentional exposures to toxic levels of acetaminophen are common.[4]

The toxicity of acetaminophen is due to the highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). At therapeutic doses, acetaminophen is mostly converted to pharmacologically inactive glucuronide and sulfate, with only 5−10% being oxidized to the toxic metabolite, mainly by cytochrome P450 (CYP) isozyme 2E1.[2] At these concentrations, NAPQI is immediately inactivated by conjugation with glutathione and excreted in urine.[5] Toxic doses of acetaminophen cause a higher proportion of the drug to be metabolized into NAPQI. The excess NAPQI depletes the glutathione detoxification pathway after which it starts to form protein adducts through binding to cysteine groups of cell proteins.[2] This may ultimately lead to liver cell necrosis and acute liver failure.[2] The cellular damage has been found to be directly related to the dose of acetaminophen.[3]

Acetaminophen poisoning can be effectively treated with the glutathione precursor *N*-acetylcysteine. Unfortunately, acetaminophen poisoning shows few and nonspecific symptoms in the first 24 h.[3,6] Furthermore, the *N*-acetylcysteine treatment is most effective when initiated within 8−12 h after exposure,[5] and after 15 h, the efficacy of the antidote rapidly diminishes.[6] For these reasons, the National Academy of Clinical Biochemistry has endorsed screening for acetaminophen in all emergency department patients who present with intentional drug ingestion.[5] Diagnosis of acetaminophen overdose is

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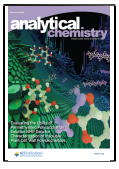

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usually carried out by determining the acetaminophen serum concentration.[7] The Rumack–Matthew nomogram, which plots the acetaminophen concentration as a function of time postingestion, is helpful in determining the likelihood of hepatotoxicity. Serum levels at or above 200 μg/mL (1.323 mM) at 4 h postingestion and 6.25 μg/mL (43.1 μM) at 24 h postingestion have been found to consistently predict hepatotoxicity. The line between these points is referred to as the probable toxicity line. The FDA later required the addition of a line 25% below the original line to build in some additional safety.[8]

In clinical settings, semirapid tests are usually carried out with spectrophotometric methods because of relative simplicity and low cost.[9,10] Despite these advantages, this method is still confined to specialized laboratories and is poorly suited for point-of-care (POC) testing. Moreover, interference causing both falsely high and low results has been reported with these methods.[9−11] In addition, competitive lateral flow immunoassays are also available for the qualitative determination of acetaminophen. These tests are, however, not quantitative, and due to high cutoff concentrations, false negatives have been reported.[12] Therefore, the development of a highly mobile, simple, and quantitative POC assay for screening of acetaminophen poisoning would be highly beneficial.

Electrochemical detection is relatively simple and inexpensive, and the required instrumentation is readily available and portable. Moreover, the use of screen-printed electrodes enables highly sensitive detection from small microliter sample volumes with little or no pretreatment.[13] Moreover, capillary blood from finger-prick collection has been previously shown to be a reliable sampling matrix for the evaluation of the pharmacokinetic parameters of acetaminophen.[14,15] This further highlights the suitability of electrochemical methods for POC screening of acetaminophen poisoning.

Low detection limits in the nanomolar range have been achieved with several novel electrode materials.[16−25] Unfortunately, interference from especially ascorbic (AA) and uric acid (UA) has been reported at concentrations expected for biological samples.[16,26] Studies have shown that the oxidation potential of acetaminophen is highly dependent on surface chemistry[27] and there is considerable variation in the oxidation potential of acetaminophen at different electrodes[18,20,22,28] in the literature. When selectivity is achieved by an anodic shift of the oxidation peak, however, interference from other endogenous chemicals is expected.[29,30] Recent reports have shown selective detection of acetaminophen in urine samples and blood serum. These reports, however, also relied on sample processing including liquid–liquid extraction, precipitation of proteins, and centrifugation as well as considerable dilution of the sample matrix.[19,20] Moreover, Moghaddam et al.[20] reported significant interference in the unextracted urine sample.

Direct electrochemical detection of acetaminophen from untreated plasma or whole blood samples has not been reported. This is likely due to both the interference from electrochemically active biomolecules and electrode fouling by proteins. We have recently shown that by combining carbon electrodes with thin-film Nafion coatings, we can virtually eliminate the interference from anions, such as AA and UA, and significantly reduce the matrix effect in the electrochemical determination of opioids in human plasma.[29,30] Moreover, these results were obtained after only a simple dilution of the plasma, without precipitation of proteins. Several other works have also reported sensitive detection of acetaminophen with Nafion-containing composite electrodes.[24−26,31,32] Most of these electrodes, however, do not show permselective properties,[26,31,32] or the interference of AA and UA was not studied.[25]

In recent years, single-walled carbon nanotubes (SWCNTs) have attracted a great deal of attention due to their unique structure and extraordinary properties, such as large surface area, mechanical strength, high electrical conductivity, and electrocatalytic activity.[33] It has been shown that SWCNT network electrodes have low charging current and enhanced mass transfer, enabling a high signal-to-noise ratio in electrochemical detection.[34] By means of aerosol chemical vapor deposition (CVD), large areas of porous SWCNT electrodes with high conductivity and surface area can be produced. This enables the production of inexpensive disposable SWCNT electrodes on a wide range of substrates including polymers. Carbon nanotube-modified screen-printed electrode strips have also been previously reported.[35−38] The fabrication of such electrodes, however, requires preparation of special carbon nanotube composite ink formulations or modification of the electrodes after screen printing. In contrast, the dry transfer process directly produces a high-surface-area electrode with high conductivity. This process allows for the collection of patterned networks that can easily be press-transferred to produce electrodes without the need for modification of conventional carbon electrodes.[39−41] Alternatively, SWCNT thin films can be laser-patterned down to 10 μm by laser ablation without any damage to polymer substrates. This process can be performed at high throughputs and is fully roll-to-roll compatible.[42]

Despite the recent increase in the availability of screen-printed electrodes, the challenge of fabricating inexpensive, durable, and reliable miniaturized reference electrodes has limited their industrial applicability.[43] Usually, screen-printed Ag or Ag/AgCl electrodes give satisfactory performance in voltammetric electroanalysis. In the screen-printing process, a layer of Ag is usually first screen-printed followed by an AgCl layer printed with AgCl ink. It has previously been shown that Nafion coatings can be used to stabilize the potential of Ag/AgCl electrodes.[44,45] Therefore, we fabricate Ag reference electrodes, without the second screen-printing step with AgCl ink.

In this work, we present a simple fully industrially compatible process for the production of disposable electrochemical test strips. We combine dry transfer and laser ablation patterning of SWCNT with screen printing of silver and slot-die coating of Nafion to realize highly repeatable electrodes with a significantly reduced matrix effect in plasma and whole blood. All of the used techniques are industrially mature and fully compatible with roll-to-roll processing. We show the determination of physiologically relevant concentrations of acetaminophen from only mildly diluted plasma and whole blood samples obtained by a finger prick.

EXPERIMENTAL SECTION

Production of Sensor Strips. The fabrication process of the disposable electrodes is described in detail in the Supporting Information, and a step by step fabrication scheme is shown in Figure S1. Briefly, the SWCNTs were first grown by aerosol CVD, as discussed in detail in refs 40, 46, and collected on a filter. An 18 × 26 cm^2 SWCNT network was then press-transferred onto an A4 PET sheet, densified, and baked for 10 min at 100 °C. The press-transferred SWCNT

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had optical transparency of 71.6% (550 nm) and a sheet resistance of 73 Ω/sq. SWCNT electrodes made with the same process have previously been characterized in detail in refs 29, 34. Silver was screen-printed directly on top of the SWCNT to fabricate reference electrodes and contact pads. SWCNT electrodes were patterned with a nanosecond pulse laser at 1064 nm wavelength. Finally, the A4 PET sheet was coated with 117 Nafion (Sigma-Aldrich), diluted to 2.5 wt % with ethanol, with a slot-die coater (FOM Technologies). Prior to measurements, strips were cut out and covered with a poly(tetrafluoroethylene) (PTFE) film (Saint-Gobain Performance Plastics CHR 2255-2) with a prepunched 6 mm hole.

Characterizations. The thicknesses of the Ag reference electrode and the SWCNT/Nafion layer were measured with a scanning electron microscope (SEM). Before imaging, cross-sectional samples were prepared with focused ion beam (FIB) milling. Both FIB milling and SEM imaging were carried out with a FEI Helios NanoLab 600 dual-beam system. Before milling, the samples were coated with 100 nm gold by evaporation (InstrumenttiMattila). The cross sections were milled with 16 kV acceleration voltage in rough milling and 280/460 pA currents. The thickness of the silver lines was also measured with a profilometer (Dektak 6 M) over several places of the lines and over the reference electrode.

Electrochemical Measurements. All measurements in a conventional 50 mL cell were carried out with a Gamry Reference 600 potentiostat. A three-electrode setup with a Pt wire counter electrode and a Ag/AgCl [sat. KCl] (Radiometer Analytical, +0.199 V vs standard hydrogen electrode) reference electrode placed in a Luggin capillary was used to measure the potential of the Ag electrode. For the cyclic voltammetry (CV) measurements performed in the 50 mL cell, the integrated electrodes of the test strips were connected. In these measurements, a modified serial ATA cable was used as a connector. All differential pulse voltammetry (DPV) and CV experiments with 40 μL drops were carried out with a PalmSens4 portable potentiostat to simulate a full POC setup.

KCl solutions with different concentrations were prepared by dissolving KCl (Merk Suprapur) in deionized water (18.2 MΩ−cm) to study the susceptibility of the Ag reference electrode to the Cl^- concentration. Morphine hydrochloride for interference studies was obtained from the University Pharmacy, Helsinki, Finland. All other chemicals were obtained from Sigma-Aldrich. For studying electron transfer, a 1 mM solution of the outer sphere redox probe $Ru(NH_3)_6$ was prepared in 1 M KCl and phosphate-buffered saline (PBS). The acetaminophen and interferent solutions were prepared in a pH 7.4 phosphate-buffered saline (PBS) solution. For the plasma measurements, expired human plasma (Octaplas AB, Sweden) was received from the blood center of Helsinki University Hospital Laboratory Services, HUSLAB (Finland). The plasma samples were diluted with a 1:1 ratio by adding 1 mL of plasma in 1 mL of pH 7.4 PBS. Whole blood was obtained by a finger prick from a healthy volunteer (plasma samples from a different person) and collected in 20 μL calibrated microcapillary tubes (Drummond Scientific Company). The blood samples were then placed in a 2 mL Eppendorf and diluted with 20 μL of PBS. The plasma and whole blood samples with acetaminophen were prepared by spiking the PBS used for dilution with twice the target acetaminophen concentration. To avoid clotting, a new whole blood sample was obtained for each measurement. For each measurement, a 40 μL drop was placed on the test strip with a micropipette. An accumulation time of 2.5 min was used for all measurements. Between measurements, the measured drop was wiped with tissue paper and the test strip was rinsed with a PBS drop for 2.5 min before the next drop was placed on the strip.

RESULTS AND DISCUSSION

Characterization. Figure 1 shows the cross-sectional images acquired from milled areas of (A) the working electrode and (B) the reference electrode. The overall thickness of the SWCNT/Nafion layer of the working electrode can be seen to be approximately 170 nm. A dark layer with a thickness of 65−75 nm between the SWCNT/Nafion layer and the Au coating can also be observed likely due to Nafion. The observation of an SWCNT/Nafion composite layer is in agreement with a previous study by us,[29] suggesting that the SWCNTs are Nafion-functionalized. The cross section of the Ag reference electrode shows flat elongated Ag particles in the few micrometer size range. Thicknesses between 5.9 and 7.2 μm were obtained for the cross sections of the reference electrodes. Similarly, measurements in different spots of the silver lines with a contact profilometer gave thicknesses in the range of 5.5−7 μm. Due to the large roughness, a clear layer of Nafion cannot be discerned on top of the Ag particles.

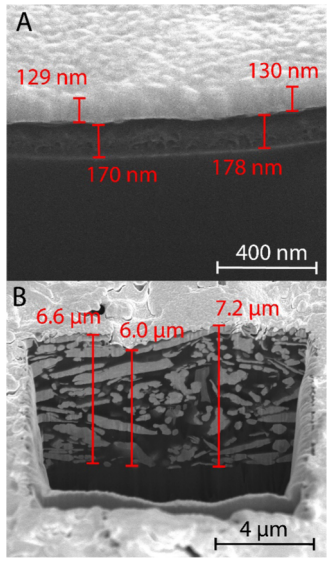

Figure 1. Focused ion beam milled cross sections on the Nafion-coated (A) working and (B) reference electrodes imaged with scanning electron microscopy.

Electrochemical Measurements. *Performance of Screen-Printed Ag Quasi-Reference Electrodes.* Usually, quasi-reference electrodes suffer from drifting potentials during measurements, short lifetimes, long run-in times before the potential stabilizes, and relatively short shelf lives.[43,47] Figure 2A shows the potential vs an Ag/AgCl electrode of both the uncoated and Nafion-coated Ag reference electrodes in a 0.1 M PBS solution. Both types of electrodes start at a potential of 84 ± 1 mV. The potential of the uncoated electrode, however, drifts significantly during the measurements and stabilizes only after approximately 2 h. In contrast, the Nafion-coated electrodes immediately show a stable potential with no required run-in time. One Nafion-coated electrode was also

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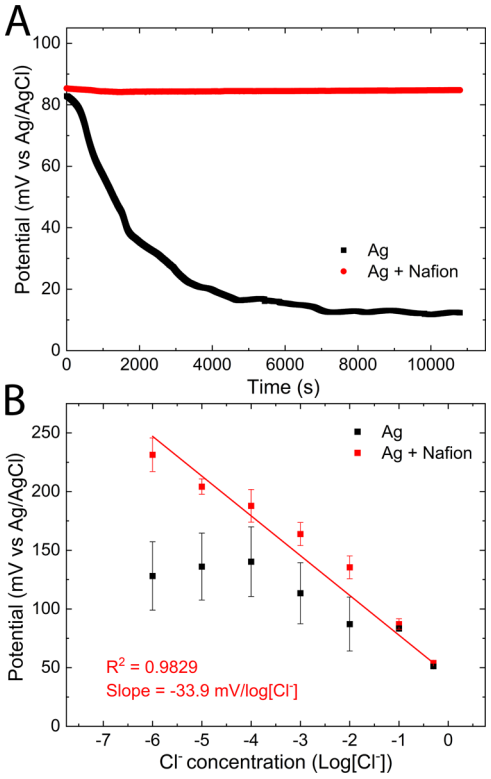

Figure 2. (A) Potential of the uncoated and Nafion-coated Ag quasi-reference electrodes vs. Ag/AgCl [sat. KCl] in 0.1 M PBS solution. (B) Potential as a function of the Cl^- concentration in KCl solutions. The error bars show the standard deviations of measurements with three different electrodes.

measured for 7.5 h giving a potential of 84.78 ± 0.35 mV. At no point during the measurement did the potential change more than ±1 mV as the lowest and highest measured potentials were 84.07 and 85.39 mV, respectively. A long-term stability study was also carried out, where a potential drop of only 9.85 mV was observed over 7 days of immersion in PBS. The potential stability and drift rate are comparable to those of screen-printed Ag/AgCl electrodes with much more complicated design with protective layers incorporating a salt matrix (KCl).[47]

Nolan et al.[45] have previously used Nafion membranes to stabilize the potential of screen-printed Ag/AgCl electrodes. Moussy et al.[44] have also shown that the potential of a Nafion-coated Ag/AgCl electrode remained constant for up to 2 weeks after implantation in a rat. In their study, however, it took 30–35 min for the potential to stabilize. In contrast, the electrode in this work immediately produces a stable potential and remains stable for up to 7 days. These measurements clearly show that the Nafion-coated electrodes can be used for voltammetric measurements in POC applications without any preconditioning. Moreover, one of the four measured electrode strips was from a different batch that was stored under ambient conditions for approximately 1.5 years prior to measurements. This electrode also showed a stable potential of 84.42 ± 0.47 mV during a 3 h measurement, indicating excellent shelf life of the reference electrode without any packaging of the electrode.

The susceptibility to Cl^- concentration was studied by measuring the potential in KCl solutions with different concentrations. Figure 2B shows the average potential of three uncoated and Nafion-coated reference electrodes (5 min measurement) in different Cl^- concentrations as a function of the logarithm of the Cl^- concentration. The uncoated

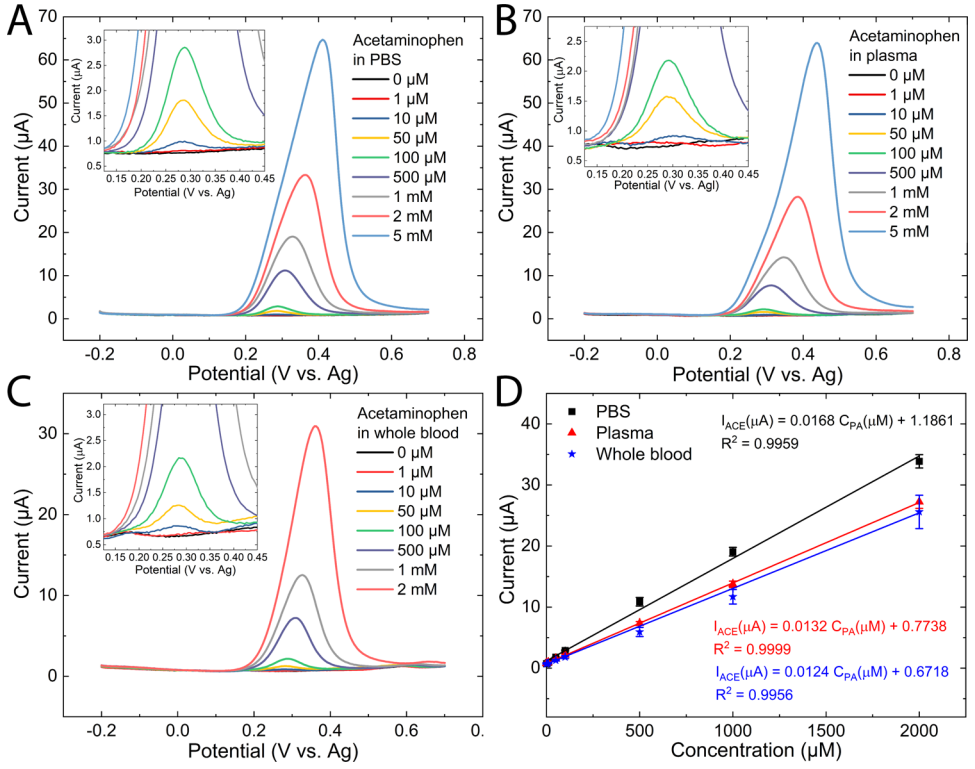

Figure 3. DPVs of increasing concentrations of acetaminophen in (A) PBS, (B) human plasma, and (C) whole blood. (D) Linearization of results in all measured matrices. The error bars show the standard deviations of four measurements with different electrodes.

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electrodes show a relatively large variation likely due to the potential drift between the measurements expected based on the data in Figure 2A. The potential of the Nafion-coated Ag reference electrodes depends linearly on the logarithm of the Cl^- concentration of the electrolyte with a slope of −33.9 mV/log[Cl^-]. The potential of the noncoated electrode shows less dependence on the Cl^- concentration. While the susceptibility of the Nafion-coated electrode is lower than that predicted by the Nernst equation for an Ag/AgCl electrode,[47] these results suggest that the use of the test strip is limited to applications where the sample Cl^- concentration is known. For this reason, all dilutions are carried out with PBS to avoid changes in the ionic strength.

Electron Transfer. The electron transfer was studied with the outer sphere redox probe $Ru(NH_3)_6$. Figure S2 shows the CV measurements with various scan rates in 1 mM $Ru(NH_3)_6$ in 1 M KCl with the Nafion-coated electrode. A peak potential separation (ΔE_p) of 68.8 mV (scan rate, 100 mV/s) was obtained, indicating close to reversible electron transfer. The increasing peak potential separation with an increasing scan rate (110 mV with 400 mV/s), however, indicates quasi-reversible electron transfer. Uncompensated resistance values of 164.1 ± 25.6 Ω were also measured for six electrodes with a 40 μL PBS solution drop.

Single Drop Analysis of Acetaminophen. Figure S3 shows the DPV measurements carried out with the sensor strip in a conventional 50 mL electrochemical cell and with a 40 μL drop placed directly on the sensor. Background-subtracted oxidation peak currents of 1.178 and 1.159 μA were measured for 50 μM acetaminophen in the 50 mL cell and with a 40 μL drop, respectively. Figure S4 shows DPV measurements with different pulse amplitudes with a 40 μL drop of human plasma. A larger pulse amplitude expectedly leads to an improved sensitivity toward acetaminophen with only a negligible increase in the background peaks at around 150 and 550 mV. This indicates an improved selectivity toward acetaminophen. Because the best signal-to-background-current ratio was observed with a pulse amplitude of 75 mV, this pulse amplitude was chosen for all further measurements.

Figure 3 shows the DPV measurements with increasing acetaminophen concentrations in PBS, plasma, and whole blood as well as the linearization of the currents. The oxidation currents scale linearly with the concentration in the range of 1 μM to 2 mM. Correlation coefficients of R^2 = 0.9959, R^2 = 0.9999, and R^2 = 0.9956 were obtained for PBS, plasma, and whole blood, respectively, indicating high linearity throughout the physiologically relevant concentration range.[7] The limit of detection (LOD) was calculated as LOD = $(3 \times \sigma)/S$, where σ is the standard deviation of three measurements in blank PBS and S is the sensitivity over the whole linear range. The LOD was determined separately for four electrodes giving an average value of 0.819 ± 0.265 μM. The highest LOD was 1.06 μM, still well below the cutoff concentration used by most clinical laboratories of approximately 66.15 μM (10 mg/L).[12] The results show that the developed test strip can quantitatively determine the blood acetaminophen at these levels even after dilution with PBS and taking into account the lower recovery in plasma and whole blood.

Based on the slopes in Figure 3D, recoveries of 79 and 74% were obtained in plasma and whole blood, respectively. The relative standard deviations (RSDs) of the oxidation currents over the whole linear range were 4.3, 7.0, and 10.0% in PBS, plasma, and whole blood, respectively. It should be noted that the used plasma and whole blood were from different individuals. It should further be noted that the whole blood measurements were carried out on three separate days and at different times of the day without fasting. Due to the larger variation in the plasma and whole blood measurements in Figure 3, a recovery test with single measurements of spiked whole blood samples at three different concentrations was also carried out with three electrodes at each concentration. The results are presented in Table 1, showing recoveries of around

Table 1. Recovery Study in Whole Blood[a]

added	found	recovery %	RSD % (n = 3)
50	36.5	73.1	7.4
100	74.7	74.7	5.5
500	371.8	74.4	1.9

[a]Average of three determinations with three different electrodes.

74%. RSD values of 7.4, 5.5, and 1.9% were obtained at concentrations of 50, 100, and 500 μM, respectively. It should be noted that these results were obtained by drawing finger-prick whole blood, diluting with an acetaminophen-spiked PBS solution, and transferring the sample onto the test strip. The RSD values therefore represent the cumulative error from all of these steps.

Protein-bound fractions of 20−25% have been previously reported for acetaminophen.[48] The unbound fraction was also found to be independent of concentration in the clinically relevant concentration range. Similar results were also obtained in a recent report,[29] where we found recoveries of 61.4 and 41.5% for morphine and codeine, respectively, with a Nafion-coated SWCNT electrode. These recoveries closely match previously reported unbound fractions. Banis et al.[49] also concluded that only the free fraction of clozapine, a benzodiazepine, contributes to the measured electrochemical signal in bovine serum albumin-containing analyte solutions with a chitosan-based composite-coated electrode. These results suggest that the electrodes coated with polymer membranes can directly determine the unbound acetaminophen fraction, without the need for time-consuming equilibrium dialysis.

As can be seen from Table S1, lower detection limits and relatively wide linear ranges have previously been reported by several groups.[16−25] However, as is evident from the treatment nomogram, extreme sensitivity is not required. The works in Table S1 also rely on time-consuming sample processing, including precipitation of proteins and considerable dilution, to reduce matrix effects that significantly increase the estimated assay time. Moreover, none of them report the detection of acetaminophen in whole blood. It should also be noted that further processing is required to obtain serum and plasma samples from whole blood. In standard operating procedures, 10−15 min of centrifugation is required to obtain plasma and serum samples.[50] For example, Brahman et al.[17] centrifuged the blood samples for 30 min at 4000 rpm to obtain human serum samples. In addition, around 30−60 min is required for clotting to obtain high-quality serum samples.[50] The protein precipitation carried out by most works in Table S1 also requires further chemical treatments and centrifugation. These sample treatment steps lead to assay times in excess of 45 min and dilution of the sample by approximately two times. In contrast, the assay developed in this work can be used for the determination of the acetaminophen concentration from

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finger-prick whole blood, only after diluting with equal part PBS and without the precipitation of proteins in less than 5 min, making it the only electrode applicable in a clinical POC device.

Passivation. To verify that the lower recoveries in plasma and whole blood are not due to fouling by proteins, the passivation of the electrode was studied. First, 1 mM $Ru(NH_3)_6$ was measured in both PBS and human plasma. Figure S5 shows no apparent passivation of the electrode when 1 mM $Ru(NH_3)_6$ is measured in PBS and diluted human plasma. This result is in line with a similar passivation study carried out with a Nafion-coated SWCNT electrode in previous research.[29] Furthermore, the electrodes used to measure 50 μM acetaminophen in whole blood (see Table 1) were also used to measure 50 μM acetaminophen in PBS. After wiping away the whole blood, washing with a 40 μL drop of PBS, and confirming that the background returns to that of blank PBS, a mean peak current of 1.83 $\pm$ 0.09 μM was obtained. This represents a recovery of 101.7%, indicating that there is no permanent fouling after whole blood measurements. The passivation was further studied in high concentrations of acetaminophen, by performing 10 consecutive DPV scans in plasma and whole blood with 1 mM acetaminophen. Figure S6 shows the measured oxidation currents as a function of the scan number (no washing was carried out between measurements). These measurements gave RSDs of 3.6% in whole blood and 4.3% in plasma, indicating that no passivation occurs during 10 measurements.

Interferent Studies. The lack of any matrix effect in the background currents in Figure 3 shows that no appreciable interference is caused by endogenous substances. Despite this, other drugs may cause interference in the determination of acetaminophen. Therefore, several drugs frequently taken in concomitant overdose with acetaminophen, including NSAIDs, caffeine, amoxicillin, and opioids,[51] were tested. The opioids morphine (MO), the active metabolite of codeine and heroin, and *O*-desmethyltramadol (ODMT), the active metabolite of tramadol, have phenol functionalities and are accumulated by Nafion due to their positive charge. Therefore, these two opioids were tested for interference. In cases where high concentrations caused interference, the tolerance limit was defined as the maximum concentration of the interferent that caused an error of less than 5% in the acetaminophen determination. Figure 4 shows the DPV scan in the absence and presence of a NSAID mix (containing 100 μM ibuprofen, naproxen, and aspirin), 1 mM salicylic acid (SA), 1 mM nicotine, 1 mM amoxicillin (Amox), 1 mM caffeine (CAF), 10 μM ODMT, and 2.5 μM MO. It is evident that the NSAID mix, 1 mM SA, 1 mM Amox, 1 mM nicotine, and 1 mM CAF did not cause more than 5% interference. Much lower tolerance limits of 2.5 μM for morphine and 10 μM for ODMT were obtained. These concentrations, however, are high compared to the therapeutic levels. Even in fatal cases of morphine and tramadol poisoning, the concentrations remain below the tested concentrations at approximately 1.75 and 3.8 μM, respectively.[52]

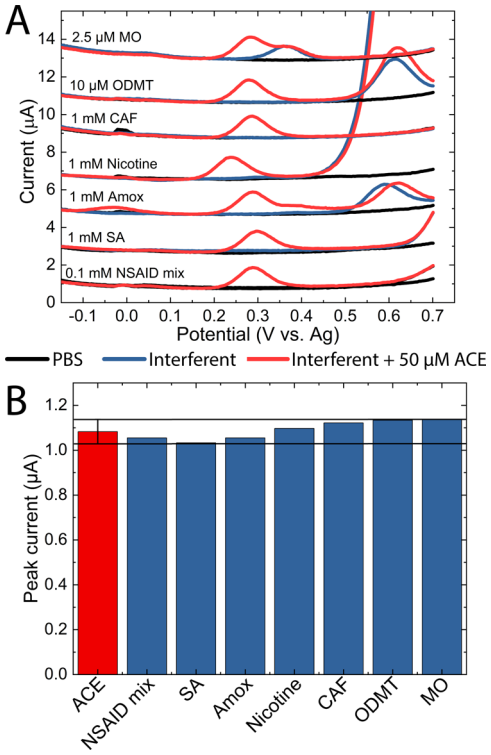

Figure 4. Interference study. (A) DPV scans in blank PBS (black line), interferent alone (blue line), and interferent + 50 μM acetaminophen (red line). (B) Background-subtracted peak current for 50 μM acetaminophen (ACE) alone (red) and acetaminophen in the presence of the interferent (blue). The error bar represents a 5% error defined as the tolerance limit. The DPV scans in (A) have been offset for clarity.

CONCLUSIONS

We show a mass production compatible fabrication process of a disposable Nafion-coated electrochemical test strip with patterned SWCNT electrodes and screen-printed silver quasi-reference electrodes for the quantitative point-of-care determination of acetaminophen. The produced silver reference electrodes show excellent shelf life, long-term stability, and short hydration time. With this test strip, clinically relevant detection limits and a linear range for determination of acetaminophen were achieved. Furthermore, the detection of acetaminophen was demonstrated in a similar wide concentration range in spiked human plasma and whole blood obtained through a finger prick. Recoveries closely match the unbound fraction of acetaminophen in blood, suggesting that the electrode is measuring exclusively the unbound fraction of acetaminophen. Aside from mild dilution, no sample treatment is required and an assay time of less than 5 min is achieved. Moreover, selectivity was also shown in the presence of several interferents. These results suggest that the developed test strip can be used as a highly portable and fast point-of-care assay for screening of acetaminophen poisoning, requiring only 20 μL of sample. However, further validation with real patient samples and parallel measurements with standard laboratory assays are still needed. Similarly, pharmacokinetic parameters will need to be evaluated from both venous and capillary finger-prick blood samples. Further development to enhance the sensitivity, without compromising the selectivity, or miniaturization of the active electrode area is also required to reduce the sample size to 10 μL or below.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01857.

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Detailed description of the test strip fabrication process, comparison of measurements in a 50 mL cell vs 40 μL drop; cyclic voltammogram of 1 mM $Ru(NH_3)_6$ in 1 M KCl, optimization of DPV parameters in plasma, passivation study with $Ru(NH_3)_6$ and acetaminophen, and comparison of electroanalytical methods for the detection of acetaminophen in biological matrixes (PDF)

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Notes

The authors declare no competing financial interest.

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